PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Docket No: **Q108487**
Francisco Javier VILA PAHI et al	Conf. No.: **8146**
Appln. No.: **10/590,311**	Group Art Unit: **1623**
Filed: **August 23, 2006**	Examiner: **Krishnan, Ganapathy**

For: **THERAPEUTIC USE FOR A GROUP OF SULPHATED POLYSACCHARIDES**

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, ANA MARIA TORRENT GIBERT (name), hereby declare and state:

THAT I am a citizen of SPAIN (country);

THAT I have received the degree of BIOLOGIST / BIOCHEMIST (degree type) in 1993 | 1995 (year) from UNIVERSITAT AUTÒNOMA DE BARCELONA (institution);

THAT I have been employed by BIOIBERICA since 1995, where I hold a position as RESEARCH SCIENTIST (job title), with responsibility for PRECLINICAL STUDIES (responsibilities);

THAT I supervised the following experiment, and have reviewed the Office Action of October 27, 2008, the specification of the present application, the claims of the present application and the prosecution of the present application.

Evaluation of the *in vivo* chondroprotective activity - Comparative study of inulin polysulphate and chondroitin sulphate

The objective was to evaluate the chondroprotective activity (cartilage protective activity) of inulin polysulphate in a model of experimental osteoarthritis induced by partial medial meniscectomy in guinea pigs (A.M. Bendele, *Progressive chronic osteoarthritis in femorotibial joints of partial medial meniscectomized guinea pigs, Vet. Pathol. 24*, 444-448 (1987)), comparing said activity with the chondroprotective activity of chondroitin sulphate.

Materials and methods

Dunkin Hartley (DH) guinea pigs with a weight comprised between 592-716 g were used. A partial medial meniscectomy was performed in a part of them.

The following treatment groups were formed: blank control group (corresponding to healthy guinea pigs), operated control group (corresponding to guinea pigs in which a partial medial meniscectomy was performed to induce an experimental osteoarthritis in them), operated guinea pigs to which the sodium salt of inulin polysulphate at 80 mg/kg of body weight/day was administered, operated guinea pigs to which the sodium salt of inulin polysulphate at 40 mg/kg of body weight/day was administered, operated guinea pigs to which the sodium salt of chondroitin sulphate at 80 mg/kg of body weight/day was administered. All the treatments were given orally.

On the day after the surgery, the daily administration of the corresponding oral treatments by means of gastric gavage was started, the carrier used being water for injection. The blank control and operated control groups only received the carrier (water for injection) by oral route.

The treatments were administered once a day for 70 days (ten weeks). The body weight and the clinical signs of the animals were recorded twice a day. No remarkable clinical signs were observed throughout the treatment period.

On the day after the administration had ended, the animals were sacrificed by anaesthetic overdose (pentobarbital sodium) and the femorotibial joint of the right rear leg was removed for its fixing in 10% formol for at least 48 hours. Then, they were decalcified, embedded in paraffin, sectioned and stained with Hematoxylin-Eosin and Safranin-O-Fast Green for histological evaluation, which was performed by a pathologist in a blind manner.

The severity of the osteoarthritic injuries was evaluated by means of the modified histologic/histochemical scale of Mankin *et al.* (H.J. Mankin and L. Lipiello, "Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips", *J. Bone Joint Surg.-Amer.*, 53-A:523-537 (1971)).

The scale evaluates the severity of the osteoarthritic injuries based on the loss of glycosaminoglycan (GAG) content, structure changes and cell changes. A total global histopathological score can be obtained by means of the Mankin scoring system, which for each animal is the sum of several parameters: cartilage structure, cellular proliferation and GAG content.

The evaluation of the amount of glycosaminoglycans (GAGs) was performed by means of Safranin O-Fast Green. The loss of red colour intensity with Safranin indicates a loss of proteoglycans, which is observed with the degenerative changes of the cartilage.

The Hematoxylin-Eosin staining is used to evaluate the changes in the structure of the cartilage and changes at the cellular level.

Outliers were excluded from further analysis according to the Grubb's Test.

Two types of evaluations of the obtained data were performed:

Chondroprotective activity

Based on the results obtained with the Safranin O-Fast Green and Hematoxylin-Eosin staining, the calculation of the chondroprotective activity was carried out.

For the calculation of the chondroprotective activity, first the index of incidence of each parameter ($I_{parameter}$) was obtained by multiplying the value of the described score (S_i) by the number of animals having such score (n_i). Since the sizes of each group are not identical, they were standardized by dividing by the total number of individuals of the group and multiplying by 100:

$$I_{parameter} = \frac{\Sigma (n_i \times S_i)}{\Sigma n_i} \times 100$$

Then, the chondroprotective activity of each treatment was calculated as the coefficient of variation from the index obtained in each of the three parameters (GAGs, cartilage structure, chondrocyte proliferation) according to the following formula:

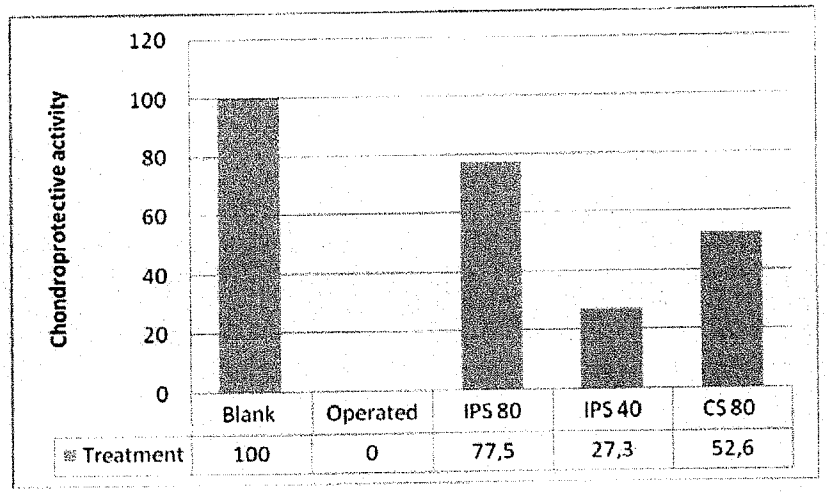
$$\text{Chondroprotective activity} = CV = \frac{I_{Operated\ control} - I_{Treatment}}{I_{Operated\ control} - I_{Blank\ control}} \times 100$$

Taking 100% as a reference for the blank control group and 0% for the operated control group, the average chondroprotective activity of the three parameters was determined.

Results

The Figure shows the chondroprotective activity of a blank control group, an operated control group, an operated group treated with inulin polysulphate at 80 mg/kg of body weight/day, an operated group treated with inulin polysulphate at 40 mg/kg of body weight/day and an operated group treated with chondroitin sulphate at 80 mg/kg of body weight/day.

As can be seen in the Figure, inulin polysulphate administered at 80 mg/kg of body weight/day showed higher chondroprotective activity than inulin polysulphate at 40 mg/kg of body weight/day. At the same dose (80 mg/kg of body weight/day), inulin polysulphate was more effective than chondroitin sulphate (superior condroprotective activity).



CS = chondroitin sulphate, sodium salt

IPS = inulin polysulphate, sodium salt

FIGURE

Evaluation by the modified Mankin method

With the scoring system, separate scores were assigned for the Safranin staining of GAGs (score of 0-6), cellular proliferation (score of 0-4) and cartilage structure (score of 0-5). Then, the scores obtained in each subcategory for each animal were added and the global histopathological score was obtained. The statistical evaluation between the different groups was carried out by means of the Mann-Whitney U test (F.J. Gravetter and L.B. Wallnau, *Statistics for the Behavioral Sciences, Wadsworth Publishing*, 7 edition (2006), see Table).

	Blank control	IPS 80 mg/kg/day	IPS 40 mg/kg/day	CS 80 mg/kg/day
Operated control	**0.001***	**0.036***	0.499	0.315

* $p < 0.05$. Mann-Whitney test

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TABLE

Results

The data in the Table confirm that there are no statistically significant differences between the chrondroprotective activity in the operated group and the group treated with inulin polysulphate at 40 mg/kg of body weight/day. The group treated with chondroitin sulphate at 80 mg/kg of body weight/day showed some improvement in chrondroprotective activity as

compared to the operated group, but the improvement was not statistically significant. In contrast, the group treated with inulin polysulphate at the higher dose (80 mg/kg of body weight/day), showed statistically significant improvement in chondroprotective activity as compared to the operated group ($p < 0.05$).

CONCLUSIONS

Treatment with inulin polysulphate at 80 mg/kg of body weight/day led to a lower incidence of injuries that was similar to the blank control group (healthy guinea pigs).

DECLARATION UNDER 37 C.F.R. § 1.132
U.S. Application No. 10/590,311 (Q108487)

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: January 27, 2009

Name: ANA MARIA TORRENT GIBERT